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Effects of Nutritional Factors on Metabolism of Dietary Cadmium at Levels Similar to Those of Man

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Several nutrients are known to affect cadmium toxicity, but little is known about the effect of dietary nutrient levels on absorption and tissue retention of cadmium at low dietary levels, similar to those of man. Feeding graded levels of zinc in a casein-gelatin diet to young Japanese quail with ^{109}Cd (as the chloride) and 0.062 ppm added cadmium decreased the cadmium concentrations in the proventriculus-ventriculus, duodenum, jejunum-ileum, and the liver, but not in the kidney. Zinc also affected some zinc, iron, manganese, and copper tissue levels. Different tissue concentration patterns of cadmium and essential minerals were obtained with two purified control diets, one based on casein-gelatin and the other on soy isolate as the principal protein sources. The data show that relatively small dietary changes can markedly affect tissue levels of cadmium and that a low intake of zinc may increase the risk to dietary cadmium exposure. The complexity of the nutrient interrelationships and their effects on cadmium require further study to define mechanisms, which may be similar to those produced by low cadmium intakes in man.

Introduction

Many investigators have shown that the nutrient composition of the diet can markedly influence the severity of toxic manifestations that occur after a few days or weeks of feeding high levels of cadmium. Single deficiencies of zinc, copper, iron, and calcium, and combined deficiencies of calcium, zinc, and protein markedly exacerbated the toxic effects of cadmium. Supplements of zinc, copper, iron (primarily the divalent form as ferrous sulfate), ascorbic acid, and L-cysteine were shown to be protective. These nutrient-cadmium interactions have been reviewed (*1–4*).

The practical problems of cadmium toxicity in man are not due to high levels of cadmium intake, but rather to very long-term cadmium accretion in the kidney until levels that can cause kidney damage occur (*5*). Experimental data that are most pertinent to man include those from studies on the effects of dietary nutrient levels on absorption and long-term retention of very low levels of dietary cadmium, similar to the intakes of man (*6*). Functional and morphological changes in the kidney under these conditions need to be investigated. The tissue retention of single oral doses of radioactive cadmium with low total cadmium intakes has been elevated by deficiencies of iron (*7, 8*), calcium (*9*), and protein (*10*). Hamilton and Smith (*11*) produced a change in the distribution of a tracer dose of ^{115m}Cd between the liver and kidneys by feeding a low calcium diet; however, calcium level had no effect on the total amount of ^{115m}Cd in the two organs.

Jacobs et al. (*12*) found that simultaneously doubling dietary levels of zinc, copper, and manganese in a soy isolate diet caused a marked decrease in the retention of ^{109}Cd (fed as the chloride to Japanese quail between 7 and 14 days of age) in the liver, kidneys, and jejunum-ileum. Cadmium in the duodenum was not affected. The total dietary levels of cadmium, 0.020, 0.082, 0.145, 0.270, 0.520, and 1.020 ppm, bracketed dietary concentrations equivalent to the intake of man (approximately 0.08–0.10 ppm), assuming the absence of moisture and fiber for similarity to the type of diet fed in the bird experiment. With both the basal and supplemented

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diets, cadmium accumulated in the duodenum, liver, and kidney in a linear log-dose, log-response relationship. Within each of the two dietary matrices, the percentage retention of the ^{109}Cd dose in each tissue was the same at each cadmium dose level. The same supplement also had a beneficial effect on the long-term whole body retention curve in accelerating the loss of ^{115m}Cd (total 1 ppm cadmium) that had been fed in the diet to Japanese quail between 7 and 14 days of age (*13*). Zinc was shown to be the primary component of the three-element supplement responsible for lower tissue cadmium concentrations following the 7-day feeding regime (*14*). Copper and manganese had small effects, which included increases in some tissue concentrations of cadmium. Jacobs et al. (*15*) showed that in birds fed a soy diet, concentrations of a dietary tracer of ^{109}Cd (total 0.145 ppm cadmium) in the jejunum-ileum, liver, and kidneys declined as dietary zinc was increased from 15 to 30 and 60 ppm in the diet; 120 ppm zinc had no further effect.

The capacity of the small intestine of young Japanese quail to accumulate cadmium results in significant concentrations of cadmium in both the duodenum and the jejunum-ileum of birds fed basal diets. The cadmium level is sufficiently high to permit assay by flame atomic absorption spectrophotometry. The high correlation between decreases in jejunal-ileal concentrations of cadmium and decreases in liver and kidney cadmium led Fox et al. (*16*) to use the concentration of cadmium in the jejunum-ileum as an index of cadmium bioavailability from human foods. With the soy isolate diet, cadmium (as the chloride) fed to give total dietary concentrations of 0.082, 0.145, and 0.270 ppm cadmium resulted in linear relationships between the logs of dietary cadmium concentrations and logs of duodenal and jejunal-ileal cadmium concentrations. The concentrations of cadmium in the two small intestinal segments were significantly lower with the casein-gelatin diet than with the soy isolate diet. The relative bioavailability value (as compared with cadmium as the chloride) for oysters was similar with the two diets, 38 ± 18% and 48 ± 13% with the soy and casein-gelatin diets, respectively. Earlier studies by Fox et al. (*17*) had shown marked differences in cadmium toxicity and tissue mineral levels when soy isolate, casein-gelatin, or dried egg white was the dietary protein source. From these varied data, it was concluded that investigations of single nutrient variables and of foods (plant and animal) intrinsically labeled with ^{109}Cd during growth are needed to identify dietary components (singly and together) that can affect accumulation of cadmium in the liver and kidneys and to establish the relationships between cadmium concentrations in the jejunum-ileum and those in the liver and kidneys.

Casein and gelatin typically contain lower levels of contaminant minerals and other complicating factors, such as phytate, and thus contribute to a better dietary matrix for studying mineral interactions than does soy isolate. Variations in mineral levels between batches are usually less for casein and gelatin than for soy isolate. The purpose of the present study was to determine the effect of variable dietary zinc concentrations in a casein-gelatin diet upon tissue cadmium levels derived from low dietary cadmium intake and to compare soy isolate with casein-gelatin as protein sources when the zinc concentrations were at the required level for each diet.

Experimental Procedures

Day-old Japanese quail (*Coturnix coturnix japonica*) of both sexes from our stock colony were housed in heated, continuously lighted, suspended stainless steel cages. Precautions to avoid environmental and dietary contamination with trace elements were observed. The birds were housed one group per cage except for birds 7–14 days of age in experiment 1, when they were housed individually to permit measurement of food intake. Diet and deionized drinking water were available at all times. The birds were wing-banded at 7 days of age and were weighed at weekly intervals. In experiment 1, groups of 10 birds each were fed with either a soy isolate or a casein-gelatin diet (Tables 1 and 2). In

Table 1. Composition of control diets.[a]

Component	Casein-gelatin diet, g/kg	Soy isolate diet, g/kg
Casein, vitamin test[b]	280	—
Gelatin[c]	70	—
Soy isolate	—	350
Glycine[d]	5	5
DL-Methionine[d]	6	6
L-Arginine · HCl[d]	6	—
Choline dihydrogen citrate[b]	8.44	6.33
Corn oil	40	40
Salts[e]	58	58
Glucose monohydrate	526.56	534.67

[a] Component sources, except as noted, and levels of vitamins as reported (*12*).
[b] Teklad, Madison, Wisc.
[c] Wilson, 2X gelatin, 75 bloom, Wilson Foods Corp., Calumet City, Ill.
[d] NRC grade, Teklad.
[e] Supplied per kg diet: $CaHPO_4$, 28.4 g; $CaCO_3$, 10 g; Na_2HPO_4, 7 g; NaCl, 4 g; KCl, 7 g; Na_2SeO_3, 0.44 mg. Reagent grade chemicals, J. T. Baker Chemical Co., Phillipsburg, N. J.; Na_2SEO_3, Alfa Inorganics, Beverly, Mass. See Table 2 for amounts of cadmium, zinc, iron, manganese, copper, and magnesium.

Table 2. Principal compositional differences between the casein-gelatin (CG) and soy isolate (S) control diets.[a]

Diet	Cd	Zn	Fe	Mn	Cu	Mg	Phytic acid
Dietary component, mg/kg diet							
CG	0.06221	20	100	2.5	1.5	300	0
S	0.07506	30	100	12	5.6	300	7000[b]
Proportion in protein, %							
CG	0.34	56	2	1	22	5	0
S	17.40	38	65	19	100	2	100
Proportion in other components, %[c]							
CG	0.00[d]	—	—	32	41	—	—

[a] The balance of noncontaminant elements was supplied by cadmium chloride, zinc carbonate, ferric citrate (16% iron), manganous sulfate monohydrate, cupric sulfate, and magnesium sulfate. Reagent grade chemicals, J. T. Baker Chemical Co., Phillipsburg, NJ, were finely ground and thoroughly premixed with glucose monohydrate. Mineral premixes and protein were assayed by atomic absorption spectrophotometry to provide precise dietary control. The amounts of minerals in each diet met the requirements with no excess (*18–20* and unpublished data). The same batches of each protein were used in both experiments. The amounts of protein per kg of diet were: casein, 280; gelatin, 70; and soy isolate, 350.
[b] Estimated from analysis of other lots of soy isolate.
[c] Amounts of components other than purified protein and chemical source of individual elements. Due to the higher dietary levels of manganese and copper in the soy diet, the small amounts of these elements in other components were not included in the dietary calculations.
[d] It was not possible to assay the calcium salts.

experiment 2, 40 day-old birds were fed the casein-gelatin diet (Tables 1 and 2) for 1 week and 20 birds were fed the soy diet. On day 7, the birds were redistributed by body weight into 4 groups of 10 birds each; 3 groups were fed the casein-gelatin diet and one group was fed the soy diet. Excess birds at the weight extremes were eliminated so that the mean body weights ± SE were 20.0 ± 0.47 g and 20.3 ± 0.30 g for the casein-gelatin and soy isolate diets, respectively. The birds continued to receive the same diets except that the zinc concentrations in the casein-gelatin diets were adjusted to totals of 12 and 60 ppm for 2 of the groups.

Accelerator-produced carrier-free ^{109}Cd (as the chloride) in 0.1*N* HCl (New England Nuclear Corp., Boston, Mass.) was premixed with glucose, freeze-dried, finely ground in a mortar, and mixed with each diet to provide 100 μCi ^{109}Cd/kg diet. The labeled diet was fed to the birds from 7 to 14 days of age. Total food intake of each bird during the second week was measured in experiment 1.

The birds were decapitated on day 14 without a prior fast. The liver was removed, washed in 0.75% NaCl solution, and blotted with tissue. In experiment 1, the intestinal tract was closed with a hemostat placed immediately distal to the ventriculus to prevent contamination of tissues with ^{109}Cd in the digesta, and the entire intestinal tract was removed. The duodenum was defined as the loop encircling the pancreas and the jejunum-ileum was the next section of small intestine terminating at the cecal juncture. The sections of small intestine were placed on pieces of rigid plastic, opened longitudinally, washed thoroughly with a stream of 0.75% NaCl solution to remove all intestinal contents, and blotted with tissue. The kidneys were blotted *in situ* to remove any blood. They were removed with bent stainless steel microspatulas with sharpened edges. All tissues were immediately placed in preweighed vials with tightly fitting caps. In experiment 2, tissues were removed identically except that the proventriculus and ventriculus were also excised, opened, and washed with saline to remove all contents.

The tissue weights were obtained and the whole tissues were solubilized in 5 ml concentrated nitric acid (redistilled, G. F. Smith, Columbus, Ohio) and diluted with deionized water to a final volume of 10 ml. Except for some residual fat, each tissue was completely solubilized. Radioactivity of the tissues and 0.5 g samples (in triplicate) of diets similarly solubilized were measured in a NaI (Tl) crystal scintillation detector (Model 5285, Packard Instruments, Des Plaines, Ill.). An integral window was employed to maximize efficiency and the tissues were counted to an error of less than 2%. All samples had identical geometry.

After measurement of ^{109}Cd in experiment 2, the solubilized tissues were transferred to large test tubes; 20 ml of a 5:1 mixture (volume:volume) of nitric and perchloric (70%, double distilled, G. F. Smith) acids were added. The tissues were wet-digested and diluted to volume. To control viscosity and minimize phosphate interference, the final solution contained 10% glycerol and 0.7% perchloric acid by volume (*21*). Half-gram samples of diet and 0.1 g samples of mineral premixes (in quintuplicate) were similarly digested. Zinc, iron, manganese, and copper were determined by atomic absorption spectrophotometry (Model 503, Perkin-Elmer Corp., Norwalk, Conn.). Cadmium in the cadmium premix and magnesium in the diets and magnesium premix were similarly determined. By these techniques, analytical values for the same elements in Bovine Liver, Standard Reference Material 1577 (National Bureau of Standards, Washington, DC) fell within the certified ranges.

Triplicate 3 g samples of casein, gelatin, and soy isolate were wet digested with 20 ml nitric and perchloric acids (5:1, volume:volume) and 1 ml concentrated sulfuric acid (G. F. Smith) and were assayed for cadmium by differential pulse anodic stripping voltammetry (*22*). The cadmium concentration of all remaining dietary components minus protein, calcium phosphate, and calcium carbonate was similarly determined. Formation of precipitates with the calcium salts prevented their analysis for cadmium. The cadmium concentrations were 37.3 ng/g soy isolate and 3 ng/g gelatin. The cadmium in casein and other dietary components (exclusive of protein and calcium salts) was below the detection limits (<3 ng/g).

The tissue cadmium concentrations derived from the diet fed between 7 and 14 days of age were calculated from the specific activity of the added cadmium, 62 μg/kg. We have repeatedly observed linear log-dose, log-tissue concentrations and identical tissue retentions of cadmium within this dietary range of cadmium fed in one of several dietary matrices (*12, 16,* unpublished data). The use of added cadmium provided a constant base level so that comparisons could be made between the two types of diets.

Statistically significant differences between group means were based on use of Student's t test (*23a*). Evaluation of response magnitude in relation to dietary zinc level was based on correlation coefficients (*23b*).

Table 3. Effect of diet on growth, cadmium intake, tissue cadmium, and cadmium retention (experiment 1).[a]

Diet	No. birds	Body weight, g	Cadmium intake, ng	Total tissue cadmium, ng[b]	Cadmium retention, %[b]
CG	8	42.4 ± 2.60	2380 ± 128	358 ± 50	14.7 ± 2.0
S	10	42.7 ± 1.82	2548 ± 146	1227 ± 78[c]	48.0 ± 1.8[c]

[a] Means values ± S.E.
[b] Based on the mean total cadmium content of the duodenum, jejunum-ileum, liver, and kidneys and intake of added cadmium (0.062 ppm).
[c] Values were significantly different from those for the CG diet, $p < 0.001$.

Results

Type of dietary protein did not affect body weight or cadmium intake (i.e., food intake) of the birds (Table 3). Birds fed the soy diet retained in the four selected tissues almost half of the cadmium consumed between 7 and 14 days of age, whereas birds fed the casein-gelatin diet retained only about one-sixth. The amounts in the small intestine were by far the largest and accounted for most of this difference (Table 4). Birds fed the soy diet had greater amounts of cadmium in the liver.

There were no effects of diet on tissue weights, except that the jejunum-ileum was significantly larger in birds fed the soy diet (Table 4). Similar results were obtained in experiment 2 (Table 5). The level of dietary zinc in the casein-gelatin diet affected neither body weight nor tissue weights.

The mineral concentrations in five tissues from birds in experiment 2 are presented in Table 6. Birds fed 20 ppm zinc were the normal requirement controls for the casein-gelatin diet. There were some differences in absolute concentrations of cadmium in some tissues between experiments 1 and 2. The relative effects of diet on cadmium in the intestinal tract segments were the same; however, the values for the liver in experiment 2 were not different from those in experiment 1. With respect to other elements, the diet did not cause marked differences except that birds fed the soy diet had markedly lower amounts of iron in the duodenum and liver, and higher amounts of manganese and copper in all tissues.

With increasing levels of zinc in the casein-gelatin diet, there were increased concentrations of zinc in

Table 4. Effect of diet on tissue weight and cadmium content (experiment 1).[a]

Diet	Tissue	Tissue weight, mg	Tissue cadmium: Cd, ng/g	Total Cd, ng	Retention, %[b]
CG	Duodenum	509 ± 28	365 ± 46	189 ± 25	7.8 ± 1.0
S		536 ± 19	1111 ± 65[d]	594 ± 37[c]	23.4 ± 1.4[c]
CG	Jejunum-ileum	580 ± 47	268 ± 47	161 ± 31	6.5 ± 1.3
S		753 ± 42[e]	832 ± 70[d]	623 ± 60[c]	24.6 ± 1.5[c]
CG	Liver	1421 ± 121	3.23 ± 0.21	4.56 ± 0.43	0.19 ± 0.01
S		1468 ± 89	4.15 ± 0.25[e]	6.07 ± 0.50[e]	0.24 ± 0.02[e]
CG	Kidneys	450 ± 34	8.76 ± 0.98	3.90 ± 0.43	0.16 ± 0.02
S		415 ± 24	7.62 ± 0.55	3.15 ± 0.30	0.12 ± 0.01[e]

[a] Mean values ± SE.
[b] Proportion of the dietary intake retained by the given tissue.
[c] Values are significantly different from those for the CG diet, $p < 0.001$.
[d] Significantly different from CG, $p < 0.01$.
[e] Significantly different from CG, $p < 0.05$.

Table 5. Effects of diet and zinc level on growth and tissue weights (experiment 2).[a]

Diet	Dietary zinc, ppm	No. birds	Body weight, g	Tissue weight, mg: Proventriculus-ventriculus	Duodenum	Jejunum-ileum	Liver	Kidneys
CG	12	10	44.8 ± 1.34	1072 ± 57	524 ± 31	593 ± 52	1557 ± 90	458 ± 30
	20	10	46.3 ± 1.21	1043 ± 53	560 ± 32	556 ± 37	1580 ± 76	481 ± 20
	60	10	45.1 ± 0.89	999 ± 40	493 ± 19	540 ± 34	1552 ± 64	472 ± 21
S	30	10	45.9 ± 1.32	1162 ± 62	562 ± 22	707 ± 33[b]	1746 ± 73	408 ± 24

[a] Mean values ± SE.
[b] Values were significantly different from those of group fed 20 ppm zinc in CG diet, $p < 0.01$.

Table 6. Effects of diet and zinc levels on tissue mineral concentrations (experiment 2).[a]

Diet	Dietary zinc, ppm	Tissue	Tissue mineral concentrations: Cadmium, ng/g	Zinc, μg/g	Iron, μg/g	Manganese, μg/g	Copper, μg/g
CG	12	Proventriculus-ventriculus	97 ± 8.6	23.7 ± 0.37[b]	32.3 ± 1.3[b]	0.57 ± 0.045	2.16 ± 0.19
	20		106 ± 8.0	25.6 ± 0.43	37.3 ± 1.7	0.59 ± 0.041	2.38 ± 0.09
	60		76 ± 3.8[b]	32.0 ± 1.31[b,c]	35.4 ± 1.4	0.46 ± 0.043[b]	2.07 ± 0.11[b]
S	30		69 ± 4.6[b]	32.7 ± 0.93[b]	31.4 ± 1.4[b]	2.43 ± 0.181[b]	4.49 ± 0.24[b]
CG	12	Duodenum	764 ± 56[b]	29.1 ± 1.31[b]	180 ± 31	1.31 ± 0.095[b]	2.89 ± 0.24
	20		394 ± 50	39.7 ± 1.37	132 ± 26	0.80 ± 0.068	3.04 ± 0.23
	60		394 ± 30[c]	52.6 ± 4.46[b,c]	98 ± 17[c]	0.91 ± 0.057[c]	3.39 ± 0.11
S	30		794 ± 74[b]	35.6 ± 2.07	41 ± 4[b]	2.53 ± 0.097[b]	5.61 ± 0.43[b]
CG	12	Jejunum-ileum	457 ± 41[b]	26.5 ± 0.60[b]	118 ± 12.2[b]	0.87 ± 0.063[b]	2.44 ± 0.16
	20		161 ± 26	33.6 ± 1.31	65 ± 8.9	0.43 ± 0.088	2.34 ± 0.10
	60		63 ± 7[b,c]	45.3 ± 3.44[b,c]	46 ± 5.1[c]	0.75 ± 0.090[b]	2.64 ± 0.13
S	30		821 ± 118[b]	31.5 ± 1.52	47 ± 2.1	2.25 ± 0.082[b]	3.95 ± 0.26[b]
CG	12	Liver	4.13 ± 0.31	20.4 ± 0.73	167 ± 15	2.29 ± 0.22	4.46 ± 0.32
	20		3.71 ± 0.30	21.3 ± 0.56	153 ± 13	1.79 ± 0.12	4.32 ± 0.19
	60		3.11 ± 0.25[c]	23.1 ± 1.05[c]	163 ± 17	1.55 ± 0.08[c]	4.56 ± 0.18
S	30		4.17 ± 0.28	20.7 ± 0.56	80 ± 13[b]	4.00 ± 0.13[b]	6.28 ± 0.47[b]
CG	12	Kidneys	8.29 ± 0.48	20.9 ± 0.66	107 ± 7	1.69 ± 0.10[b]	3.56 ± 0.20
	20		8.88 ± 0.88	20.2 ± 0.33	104 ± 3	1.33 ± 0.09	3.43 ± 0.14
	60		8.48 ± 0.49	23.3 ± 0.54[b,c]	105 ± 6	1.36 ± 0.05[c]	3.68 ± 0.09
S	30		10.50 ± 0.75	24.0 ± 0.68[b]	110 ± 8	2.89 ± 0.10[b]	4.24 ± 0.11[b]

[a] Mean values ± SE.
[b] Values were significantly different ($p < 0.05$) from those of the group fed 20 ppm zinc.
[c] Values were significantly different ($p < 0.05$) from those of the group fed 60 ppm zinc as compared with 12 ppm zinc.

all tissues (Table 6). As dietary zinc increased, there was a general decrease in cadmium concentrations for all tissues except the kidneys. These relationships are all supported by statistically significant correlation coefficients (Table 7). The linear correlations between tissue zinc concentration and tissue cadmium concentration were statistically significant only between the duodenum and jejunum-ileum. The highest correlation coefficient for tissue zinc versus tissue cadmium was obtained for the jejunum-ileum and the range in cadmium concentrations was the highest for any tissue. Since the changes in liver cadmium represented the most important effect in this study, the correlation coefficient for jejunum-ileum versus liver cadmium was calculated. It was statistically significant ($p < 0.05$).

The concentrations of iron in the two small intestinal segments were generally inversely related to dietary zinc concentration (Table 6). A sensitive relationship between zinc and manganese was observed. Except for the jejunum-ileum, there was generally an inverse relationship between tissue manganese concentration and dietary zinc level. Dietary zinc had minimal effects on tissue copper concentrations.

Discussion

Facets of the Experimental Model Pertinent to Interpretation of the Data

This experimental model was designed to obtain data that may have some significance to man, under dietary conditions that could be modified readily

Table 7. Correlations between zinc and cadmium in birds fed the casein gelatin diet (experiment 2).[a]

Variables	Tissue(s)[b]	r	p
Dietary Zn vs. tissue Cd	PV	0.449	<0.05
	D	0.618	<0.01
	JI	0.685	<0.01
	L	0.457	<0.05
	K	0.025	NS[c]
Dietary Zn vs. tissue Zn	PV	0.835	<0.01
	D	0.783	<0.01
	JI	0.826	<0.01
	L	0.455	<0.05
	K	0.557	<0.01
Tissue Zn vs. tissue Cd	PV	0.208	NS
	D	0.627	<0.01
	JI	0.695	<0.01
	L	0.106	NS
	K	0.023	NS
JI Cd vs. L Cd	JI, L	0.458	<0.05

[a] Calculations are for logs of all values; $n = 30$.
[b] PV = proventriculus-ventriculus; D = duodenum; JI = jejunum-ileum; L = liver; K = kidneys.
[c] Not significant.

with respect to the level of specific nutrients and the feasibility of adding conventional human foods in future experiments. Cadmium was administered by feeding at levels comparable to those in the diet of man (*6*). Essential nutrients were present at levels required by the quail insofar as they are known. Excesses of essential nutrients were avoided insofar as possible and no substances were added to the diet that were not required.

Requirements for the quail have been determined by feeding the same graded dietary levels of a mineral for either 2 or 4 weeks. It was found, however, that the minimal level required after one week was less than that required during the first week, as the growth data in Table 5 illustrate for zinc. By lowering the dietary zinc from the required level of 20 ppm by 40%, to 12 ppm, growth and gross development during the second week were normal. This is the minimal adequate level of zinc for the second week. It is likely, therefore, that as the normal growth rate slowed during the second week, the levels of other nutrients were present in excess of requirements. Changes in nutrient requirements for human beings have been established in relation to age and growth rates (*24*); however, requirements for experimental animals have been limited to one set of requirements for the growth period (*25*). The practice of determining animal requirements by stepwise doubling the nutrient concentration in the diet and forcing maximal weight gain may also have resulted in establishing requirements that were relatively higher than the requirements set for man. These traditional types of control diets for animals have probably influenced experimental results to indicate lesser problems with cadmium than would be true for human populations, whose nutrient intakes may not exceed or even meet requirements (*4*).

The zinc levels were selected to fall within the requirement range. A lower level of zinc, which would have decreased growth rate, was avoided so that tissue concentrations of cadmium could be compared between groups without the need to obtain data on individual diet consumption. This permits the study of larger numbers of variables per experiment; however, an investigation of the effects of deficiency, either before or during the test period, would require a different model. The highest level of zinc was the same as had provided protection against low levels of cadmium fed in the soy diet (*15*). It does not represent an impossibly high level, as related to requirement, in terms of supplements for human beings under unusual circumstances of high cadmium exposure.

The tissue concentrations of cadmium at 14 days of age result from a spectrum of metabolic processes that probably proceeded at different rates during the final 7-day period. Most of our data include changes that seem to be due to nutritional effects upon intestinal absorption of cadmium. With higher cadmium levels, feeding cadmium for 48 hr immediately prior to killing at 14 days of age resulted in a high cadmium uptake by the duodenum and marked decreases in duodenal iron concentrations (*26*), and morphological abnormalities of the villi (*27*). It is thought, therefore, that changes at the intestinal level occurred early in the week of these experiments. Since ^{109}Cd was fed for the last 7 days of this experiment, the distribution of ^{109}Cd among the various organs, particularly that in the liver and kidneys, is the sum of cadmium consumed at different times and of metabolic and transport processes that have not been defined with respect to time.

Effects of Dietary Zinc on Tissue Cadmium Levels

It is significant that supplemental zinc protects against dietary cadmium over a range of intakes from less than 0.1 ppm in this experiment to 80 ppm in the first study by Supplee (*28*). High levels of cadmium interfere with zinc absorption and produce signs of deficiency; however, the levels of cadmium of importance to man are far below the levels that interfere with zinc as an essential nutrient. More subtle interactions at binding sites involved in transport and residence in tissues are undoubtedly involved in the latter case.

The best dose-response to zinc was found in the jejunal-ileal section of the small intestine. Decreases of cadmium in this section have been correlated with decreases in the liver and/or kidney (*12*, *15*). The greater difference between cadmium concentrations in the jejunal-ileal section from birds fed 12 versus 20 ppm zinc, as compared with 20 versus 60 ppm zinc, may be indicative of a more significant effect of zinc at deficient than at excess levels. This places emphasis on correcting zinc deficiency in human beings exposed to typical background levels of cadmium rather than administering zinc in excess of requirement, where safety of the zinc supplements is not well defined. In other persons exposed to high levels of cadmium, either industrially or via environmental contamination, supplements of zinc in excess of requirement should be useful. A controlled study of zinc supplements in such a population is needed.

As zinc concentrations in the diet increased, not only did zinc concentrations in the tissues increase, but the concentrations of iron, manganese, and copper decreased in one or more tissues. Single supplements of manganese and copper had some small effects in increasing tissue cadmium concentrations from low dietary intakes (*14*). It is not known what effects deficiencies of these two elements might have upon uptake under these conditions. Flanagan et al. (*8*) showed that low iron status, as indicated by low serum ferritin values, was associated with high absorption of 25 μg cadmium with ^{115m}Cd as the chloride consumed in a single meal by human volunteers (*8*). Flanagan et al. (*8*) also showed that in mice a tracer of ^{109}Cd as the chloride given with 1.12 ppm cadmium in the drinking water was bound primarily to larger proteins in the liver and kidneys rather than to metallothionein. With a high intake of cadmium the reverse distribution has been found. Additional studies are needed on the absorption and metabolism of low levels of cadmium, and the manner in which cadmium is influenced by other essential elements, both individually and interacting with each other. From a practical viewpoint, differentiation is needed between the effects of mineral status (deficient and excess) and the mineral levels consumed directly with cadmium.

Effects of Diet Type on Tissue Cadmium Levels

The lower levels of cadmium in the duodenal and the jejunal-ileal sections of birds fed the control casein-gelatin diet (20 ppm zinc) were not associated with similarly lower levels of cadmium in the liver as compared with birds fed the soy diet. Although increasing the zinc content of each diet decreased cadmium in both the jejunum-ileum and the liver, the use of cadmium content of the jejunum-ileum as an index to bioassay cadmium in natural foods now appears equivocal because of the differences between the two types of control diets. It is possible, but unproven, that given dietary supplements would influence cadmium uptake by the jejunum-ileum and liver similarly with each diet. This was true for jejunal-ileal cadmium uptake from oysters (*16*). The correlation in this study of jejunal-ileal cadmium concentrations with liver concentrations supports the validity of the approach. The range in liver values with the graded zinc levels was relatively small (Table 6), much smaller than would be expected in a bioassay with cadmium fed at three levels (*12*); therefore, correlations of cadmium in the two tissues in a bioassay range should be good.

The differences in jejunal-ileal weight and the concentrations of essential minerals in intestinal tissue all may have affected tissue cadmium levels apart from characteristics of the dietary proteins. Table 2 shows that much higher proportions of dietary iron, manganese, and copper were supplied by the soy protein than by the casein-gelatin. Although total dietary levels of each element except copper were established at levels to meet requirements with no excess, the relative decreases in requirement during the second week may have been different for each diet. Phytate in soy may affect cadmium directly or indirectly by its effect on zinc. Some more minor components of the soy isolate may also be involved.

The complexity of the apparent interrelations among the essential elements themselves, and particularly in relation to cadmium, makes one cautious in drawing conclusions regarding the best dietary mineral levels to minimize adverse effects of cadmium. The relative hazard of cadmium in an individual food must still be evaluated on the basis of total cadmium content until data are obtained on the bioavailability of cadmium in human foods.

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